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A Comparison of Bursting Neurons in *Aplysia*

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Abstract. Five types of bursting neurons have been described in *Aplysia:* three types of individual bursters—the LUQ cells, L10, and R15, plus two types of population bursters—the bag cells and the R25/L25 cells. Individual bursters can burst without any synaptic input, while bursts generated by the population bursters are shaped largely by their synaptic interactions. In this paper we review what is known about the burst mechanisms of these five classes of neurons and attempt to relate them to the roles of the five cell types in the control of autonomic function.

Introduction

Molluscan neurons that have endogenous burst generating capabilities are useful experimental preparations for the study of burst generation and modulation. This is particularly true for *Aplysia.* Because the neural circuitry of *Aplysia* has been studied extensively, one can attempt to relate the functional properties of bursting neurons to their roles in the control of behavior, and to begin a comparative study of different types of bursters within the same organism. In this paper we will compare and contrast the burst mechanisms and functional roles of five different types of bursting neurons, all of which are found in the abdominal ganglion, and all of which have unique properties: the bag cells, the R25/L25 cells, cell L10, the LUQ cells, and cell R15. Particular attention is given to R15, which has been the focus of our recent studies. The interactions between these five classes of bursting cells, as well as some of their outputs, are shown schematically in Figure 1.

The Bag Cells

The neuroendocrine bag cells consist of two symmetrical clusters of about 400 neurosecretory cells each, which are strongly coupled to one another electrically. They have only one mode of firing—a synchronous population burst in all 800 cells that lasts approximately 15–30 min (Kupfermann and Kandel, 1970). This burst is necessary and sufficient to trigger normal egg laying behavior (Pinsker and Dudek, 1977; Dudek *et al.,* 1979). Oviposition is a complex, stereotyped behavior that lasts from one to a few hours and typically occurs at an interval of one or more days (Cobbs and Pinsker, 1982; Ferguson *et al.,* 1989). The physiological stimulus that triggers a population burst in the bag cells is unknown, but when the bag cells are excited experimentally, the intraburst firing frequency and the duration of the burst are independent of the intensity of the triggering stimulus (Kupfermann and Kandel, 1970). This all-or-none burst triggers the release of a dose of egg-laying hormone into the circulatory system. This hormone then initiates the release of mature oocytes from the ovotestis (Dudek *et al.,* 1980; Rothman *et al.,* 1983b). The population burst has an exceptionally long refractory period, lasting on the order of 18–24 h, which limits the rate of occurrence of egg laying (Kaczmarek and Kauer, 1983).

The all-or-none nature of the bag cell burst results from a positive feedback, reverberatory interaction within the population. In addition to releasing egg laying hormone, the bag cells also release three neuropeptides, α-, β-, and γ-bag cell peptides, which are autoexcitatory (Rothman *et al.,* 1983a; Brown and Mayeri, 1989). Even weak excitation of the bag cells can lead to an all-or-none burst, as the cells excite one-another by these slow, chemically mediated interactions. Not only do these peptides depolarize the bag cells, they also down-regulate voltage-sensitive K^+ channels and up-regulate Ca^{++} channels, rendering the cells more excitable and resulting in enhanced spike duration. These effects contribute to prolonging the burst, thus ensuring that a suprathreshold dose of egg-

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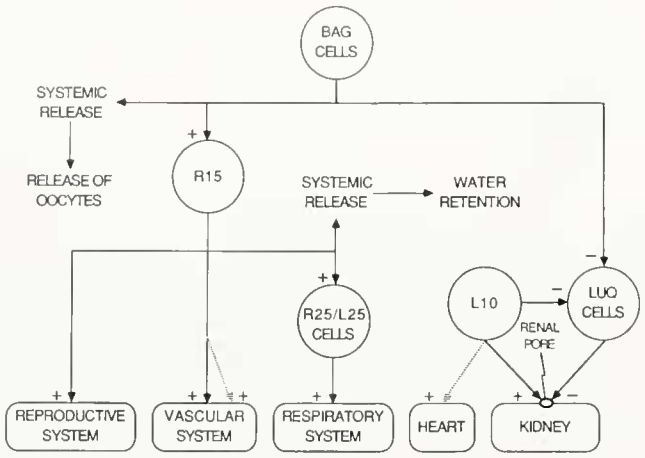

Figure 1. Summary of the interactions between bursting cells in the abdominal ganglion of *Aplysia*. The dashed lines represent indirect connections. The bag cells, L10, and the R25/L25 cells are known to make several other connections to neurons that are not shown here for simplicity (Koester and Kandel, 1977; Mayeri and Rothman, 1985; Segal and Koester, 1982).

laying hormone is released into the blood. These effects on ion channels are apparently mediated in part by activation of A-kinase and C-kinase (Strong and Kaczmarek, 1986; Strong *et al.*, 1987; De Riemer *et al.*, 1985; Conn *et al.*, 1988). An initial decrease in the levels of cyclic AMP, followed at a longer latency by a decrease in sensitivity to cyclic AMP, contribute to termination of the burst as well as to the refractory period that follows the burst (Kauer and Kaczmarek, 1985).

The R25/L25 Neurons

The R25 and L25 cells are two interconnected clusters of approximately 15 cells each, which act as trigger cells for respiratory pumping. They connect directly to the motoneurons that drive the behavior. A population burst in the R25/L25 network is necessary and probably sufficient for triggering the complete behavior (Byrne, 1983; Koester, 1989). Like egg laying, respiratory pumping often occurs episodically and in an all-or-none fashion (Pinsker *et al.*, 1970; Eberly and Pinsker, 1984). Unlike egg laying, each episode of respiratory pumping is brief, consisting of synchronous contractions of the mantle organs accompanied by heart inhibition (Pinsker *et al.*, 1970; Byrne and Koester, 1978). The motor effects typically last only 5–10 s. Individual episodes can occur spontaneously or in response to tactile or noxious stimuli (Pinsker *et al.*, 1970; Walters and Erickson, 1986). Respiratory pumping can also occur repetitively—either in a stationary rhythm with a period of a few minutes (unpub. obs.) or in a decelerating "seizure" pattern (Kanz and Quast, 1990). These repetitive episodes of respiratory pumping can occur spontaneously or in response to various environmental stimuli (Eberly *et al.*, 1981; Croll, 1985; Kanz and Quast, 1990). The functional significance of respiratory pumping appears to vary with the context in which it occurs. It has been hypothesized that respiratory pumping may function to enhance defensive withdrawal (Pinsker *et al.*, 1970), to expel defensive secretions or debris from the mantle cavity (Kupfermann and Kandel, 1969), to increase respiratory exchange (Byrne and Koester, 1978), or to contribute to the systemic circulation of hormones (Kanz and Quast, 1990).

The basic mechanism of burst generation in the R25/L25 network resembles that of the bag cells. Low frequency firing leads to a regenerative, all-or-none stereotyped burst that results from positive feedback interactions between cells in the R25/L25 network. Conventional facilitating chemical EPSPs, as well as electrical coupling, mediate these mutually excitatory connections. This positive feedback state can be accessed by two separate pathways—slow pacemaker potentials that are endogenous to the R25/L25 cells or excitatory chemical EPSPs that are generated by afferent input. Termination of the all-or-none population burst in these cells is mediated largely by synaptic interactions—slowly developing mutual synaptic inhibition and heterosynaptic depression of the mutually excitatory chemical connections (Byrne, 1983; Koester, 1989).

The LUQ Neurons

The left upper quadrant (LUQ) cells are a cluster of five similar neurons (Frazier *et al.*, 1967). A subset of the LUQ cells project to the kidney, where they ramify extensively. On the basis of their axonal projections they are thought to have extensive effects on kidney function. The only effects of these cells that have been examined in detail are on the renal pore, which they cause to close. The synaptic actions of the LUQ cells on this pore have very slow onsets and offsets, on the order of several seconds (Koester and Alevizos, 1989).

Unlike the bag cells and the R25/L25 cells, individual LUQ cells burst independently of one another. Their endogenous burst properties have been analyzed in detail (Kramer and Zucker, 1985a,b; Thompson *et al.*, 1986). The depolarizing pacemaker potential of each burst is initiated by the activation of voltage-dependent Ca^{++} channels. When the cell reaches action potential threshold, the Ca^{++} influx during each action potential causes a buildup of cytoplasmic free Ca^{++}, which has three effects. The initial effect is to activate Ca^{++}-dependent, non-specific cation-selective channels, which contribute to burst acceleration. Eventually the two slower effects of intracellular Ca^{++}-buildup predominate: (1) Ca^{++}-dependent inactivation of the Ca^{++} channels that initiated the depo-

larizing pacemaker potential leads to a phase of regenerative repolarization. (2) Activation of Ca^{++}-dependent K^+ channels also contributes to the repolarization, particularly at low temperatures.

Neuron L10

L10 also bursts endogenously (Kandel, 1976; Kleinfeld *et al.*, 1990). It is a multiaction interneuron and motoneuron that is thought to play a major role in integrating various aspects of renal function. It makes direct and indirect connections to the renal pore that oppose the synaptic actions of the LUQ cells—*i.e.*, it causes the pore to open. *In vitro* these openings occur at a rate of about one per minute. This peripheral antagonism is complemented by direct inhibitory projections from L10 to the LUQ cells. L10 also ramifies extensively in the kidney and is presumed to modulate other aspects of renal function (Koester and Alevizos, 1989). One way in which L10 may modulate renal excretion is by its excitatory connection to the heart excitatory motoneuron RB_{HE} (Koester *et al.*, 1974). The bulk filtration that gives rise to renal fluid is thought to occur within a specialized structure, the cristae aorta, which lies in series with the heart in the pericardial sac (Andrews, 1988). Therefore the increase in heart rate caused indirectly by L10 activity may increase renal filtration.

The mechanism that underlies spontaneous bursting in L10 has not been studied in detail. However, preliminary results suggest that many of the spikes that occur during a spontaneous burst are generated in peripheral axonal processes, far outside the ganglion (unpub. obs.).

Neuron R15

R15 is an endogenously bursting peptidergic neuron that is thought to play a role in integrating various aspects of egg laying. It was observed several years ago that spontaneous burst generation by R15 is enhanced by the bag cells when they fire in their population burst (Branton *et al.*, 1978). More recently, using an *in vitro* preparation, it has been found that R15 has several synaptic actions that may contribute to efficient egg laying behavior. (1) When R15 bursts spontaneously, it increases the frequency of respiratory pumping via its excitatory connections to the R25/L25 cells (Alevizos *et al.*, 1991a). (2) R15 causes contraction of the pleuroabdominal connectives by its excitatory connection to motoneuron L7 (Alevizos *et al.*, 1991b). (3) R15 increases the rate of anterograde peristalsis of the large hermaphroditic duct via its peripheral axonal processes (Alevizos *et al.*, 1991c). (4) R15 also sends processes to the left pedal-parapodial artery, by which it causes local vasoconstriction of this branch of the arterial tree (Skelton, in prep.).

It has been postulated that R15 integrates five different aspects of egg laying behavior: (1) The increase in respiratory pumping rate may enhance respiratory exchange (Alevizos *et al.*, 1991a). Alternatively, the vigorous pressure surges that occur in the arterial system as the result of gill contractions may assist in circulating egg laying hormone throughout the body (Kanz and Quast, 1990). (2) L7 is a multiaction excitatory neuron that connects to muscle in a variety of organs, as well as to neurons in the peripheral nervous system (reviewed by Umitsu *et al.*, 1987; Alevizos *et al.*, 1989). At the low rates of L7 firing elicited by R15 bursting *in vitro*, the only synaptic action that L7 expresses is excitation of the sheath muscle of the paired pleuroabdominal connectives. Each connective consists of a central axonal core surrounded by a connective tissue sheath that contains vascular channels into which the bag cells release their peptides and hormones. The accordion-like folding of the connectives in response to L7 activity may increase the fluid resistance of their vascular channels, thereby delaying the washout of the autoexcitatory peptides and ensuring that mutual excitation of the bag cells is maximally expressed (Alevizos *et al.*, 1991b). (3) The increase in peristalsis of the hermaphroditic duct presumably contributes to the mixing of the eggs with the secretory products of the duct, as well as assisting the cilia within the duct in moving the eggs to the caudal end of the genital groove (Alevizos *et al.*, 1991c). (4) The constriction of the left pedal/parapodial artery shunts arterial blood to the right pedal/parapodial artery, which perfuses the genital groove. Such an effect could help support the metabolic activity of the cilia lining the groove, which move the eggs several cm up the groove to its anterior orifice, from which they are deposited on the substrate. (5) In addition to its direct synaptic actions, R15 is also thought to have a neurosecretory action that influences water balance. R15 synthesizes R15α1 peptide, a 38 amino acid neuropeptide that causes an increase in net water retention when injected into the animal (Weiss *et al.*, 1989). R15 has numerous varicosities that appear to release into systemic vascular spaces (Rittenhouse and Price, 1985), leading to the suggestion that R15 may increase net water uptake when it is excited by the bag cells (Alevizos *et al.*, 1991c). Such an effect may be required to counter the water lost in egg formation, for the eggs are fertilized and packaged into gelatinous egg capsules on demand—*i.e.*, in response to the bag cell burst (Thompson, 1976). It will be necessary to record R15's firing pattern during spontaneous egg laying in the intact animal to determine the actual contributions of these different effects of R15 activity to egg laying behavior.

Each of the four direct synaptic actions of R15 can be mimicked by R15α1 peptide, and the peptide probably mediates them when R15 bursts. These synaptic actions are unusual in that they decay quite rapidly with repeated

activation of R15. An example of this synaptic decrement is shown in Figure 2, for the R15-R25/L25 connections. Prolonging the R15 burst period to greater than 10 min has no added effect on the excitation of the R25/L25 cells. The fact that the response of the R25/L25 cells to direct application of R15α1 peptide decreases in a similar fashion argues against depression of release being critical for the decrement in synaptic transmission. In addition, the R25/L25 cells respond normally to another excitatory transmitter when the response to R15 is depressed, ruling out non-specific refractoriness or postsynaptic inhibition as contributing to this synaptic decrement. Thus, postsynaptic desensitization appears to be the most likely explanation for the decay of R15's direct synaptic actions on the R25/L25 network. A similar conclusion is drawn from the actions of R15 on L7, on the hermaphroditic duct and on the arterial muscle. However, in the case of the two peripheral tissues, one cannot rule out muscular fatigue as a contributor to response decrement (Alevizos *et al.*, 1991c).

The direct synaptic actions of R15 are difficult to observe *in vitro* without taking special precautions. They are normally chronically depressed by the profound desensitization that results from the fact that R15 fires spontaneously at a high rate *in vitro*. Only if R15 is silenced by injecting hyperpolarizing current for 1–2 h does the desensitization decay, unmasking the four synaptic actions described above (Alevizos *et al.*, 1991a,b,c). This observation has two important implications. First, the synaptic actions of other spontaneously active neurons may be masked if they undergo profound depression. It may be necessary to silence such cells for a long time to restore their synaptic connections to a level where they can be detected. Second, the observation that R15's synaptic actions rapidly become completely depressed results in a paradox. How can these actions ever be expressed, given that R15 bursts continuously in *in vitro* experiments? Are its synaptic connections constantly desensitized? This question was addressed in chronic recording experiments, in which the axon of R15 was recorded from in intact, freely moving animals (Alevizos *et al.*, 1991a). It was found that R15 does not burst spontaneously in the intact animal (Fig. 3). Given that R15 is inactive in the intact animal and is excited by the bag cells *in vitro*, it has been suggested that R15 is a conditional burster that is switched to the bursting mode by the bag cell burst that triggers egg laying (Alevizos *et al.*, 1991a). Although preliminary results support this hypothesis, it has not yet been determined whether R15 fires during a spontaneous egg laying episode in the intact animal.

The mechanism that generates spontaneous bursts in R15 has been studied extensively (Adams, 1985; Adams and Levitan, 1985; Lewis, 1988; Thompson *et al.*, 1986). In its broad details it resembles the burst generating mechanism described above for the LUQ cells. Kramer and Levitan (1990) have demonstrated that modulation of R15 bursting by egg laying hormone is most effective when R15 is inactive, consistent with the observation that R15 is silent in the intact animal.

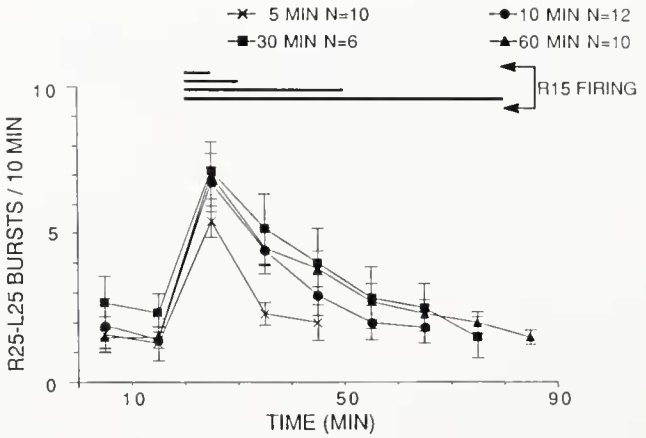

Figure 2. Modulation of the frequency of respiratory pumping by R15 decays during prolonged R15 activity. When R15 was allowed to burst spontaneously for various amounts of time after a 2-h period of hyperpolarization, it produced a long-lasting increase in the frequency of respiratory pumping. The amplitudes of the maximum effect and the time courses of decay of these increases were not significantly different for the 10-, 30-, and 60-min firing periods, while the effect produced by the 5-min firing was significantly smaller in both amplitude and duration. These data indicate that the maximum effect of R15 bursting is exerted within the first 5–10 min of R15 bursting, beyond which the response is independent of R15 activity. There was no trend for the firing rate of R15 to slow down over the course of the long burst periods (Alevizos *et al.*, 1991a).

Conclusions

It is interesting to see whether a comparison of these five types of bursting neurons leads to any conclusions about how the properties of each class relates to its functional role. Even with this relatively small sample of cell types, a few generalizations do emerge from such a comparison.

Is there a difference between individual bursters, which are not coupled to other bursting cells (L10, the LUQ cells, and R15) and population bursters, which fire as part of a population burst (the bag cells and the R25/L25 cells)? The two classes are alike in one respect—both individual bursters and population bursters can have endogenous pacemaker mechanisms (the LUQ cells, L10, R15 and the R25/L25 cells). They differ, however, in their ability to generate episodic bursts. In the *in vitro* preparations that have been examined so far, individual bursters do not seem to fire in isolated bursts. The population bursters (the bag cells and the R25/L25 cells), however, by virtue of the positive feedback chemical and electrical connec-

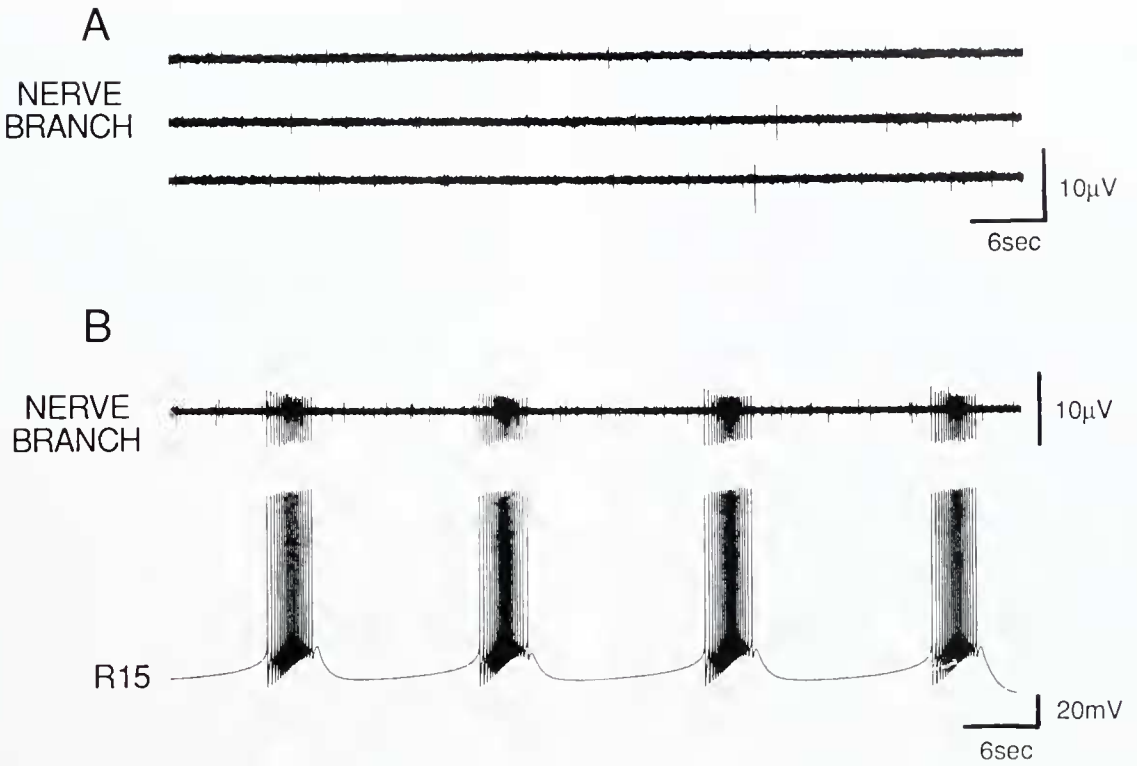

Figure 3. R15 does not burst spontaneously in the intact animal. An extracellular electrode chronically implanted in the subject was used to record activity from a small branch of the pericardial nerve that contains R15 processes. (A) In the intact animal there was no bursting activity of R15 recorded from the nerve branch. Three random 1.5-min samples are shown from a 2-h recording. (B) At the end of the experiment the animal was sacrificed, and the abdominal ganglion was dissected from the animal, along with the electrode still attached to the nerve branch. Bursting activity appeared in the nerve of the isolated ganglion. Intracellular recording from the R15 soma confirmed that the bursting activity in the nerve was due to R15 (n = 9) (Alevizos *et al.*, 1991a).

tions within each population, are well adapted to generate single bursts in response to brief volleys of excitatory synaptic input.

The excitatory and inhibitory chemical synaptic connections between members of a population of bursting cells also appear to extend the range of possible burst durations and intensities. While typical burst durations for the individual bursters are about 5–30 s (at 15°C), the bag cell bursts last 15–30 min. At the other extreme, although the burst duration of the R25/L25 network is quite variable, the high frequency terminal phase of the burst, which actually drives the motoneurons, lasts only 1–2 s. The excitatory synaptic connections between the R25/L25 cells also contribute significantly to the high firing frequencies that these cells attain during a burst—as high as 25–40 Hz. In contrast, individual bursters generally reach peak firing frequencies of only 1–3 Hz during a burst. The role of chemical connections in shaping the burst can also be extended to controlling the refractory period by the activation of second messenger systems that generate long-lasting effects. In the case of the bag cells, the refractory period can be made to last as long as 18–24 h in this way.

The shaping of the duration of the population bursts of the bag cells and the R25/L25 cells seems to have clear functional consequences. In the case of the bag cells, the long-lasting bursts with the gradual increase in spike width appear to provide a large safety margin for release of an effective dose of egg laying hormone into the circulation. For the R25/L25 cells, the very brief, high frequency burst is well suited for driving intense, synchronous contractions of the mantle organs, thereby optimizing the pumping action.

It is more problematic to understand the significance of the bursting patterns of the individual bursters. The bursts generated by one of them, L10, does have an obvious function. Each burst elicits a phasic opening of the renal pore. But whether the pore actually opens this way *in vivo* remains to be determined. In addition, it is not clear why the other two classes of individual bursters fire in a bursting mode. The synaptic actions generated by R15 and the LUQ cells are so slow that their follower

cells effectively integrate their firing patterns. That is, there is no reflection of the phasic bursting patterns of R15 and the LUQ cells on any of the nerve or muscle cells on which they synapse. This raises the question of why R15 and the LUQ cells burst, rather than firing in steady trains. Three possible explanations come to mind: (1) They may have other, more phasic synaptic actions. For example, R15 has transient synaptic actions on other neurons in the abdominal ganglion. These effects are not observed in all preparations, however, suggesting that they may be gated by some undetermined physiological variable (Alevizos and Koester, 1986; Brown and Mayeri; 1987). (2) The release properties of these cells may be such that brief bursts of activity are the most efficient for optimizing release. (3) Synaptic release in *Aplysia* is strongly influenced by the level of membrane potential immediately preceding initiation of an action potential. Hyperpolarization depresses release and depolarization enhances release (Shimahara and Peretz, 1978; Shapiro *et al.*, 1980). Perhaps the slow depolarizing waves of membrane potential that generate the bursts in these cells are conducted electrotonically to the terminals, where they may modulate spike-evoked release. However, it seems unlikely that such changes in resting potential would be conducted to terminals in the periphery. Therefore, a cell like R15, which makes both central and peripheral synapses, may have quite different release properties at its synapses within the ganglion compared to those in the periphery. If there does exist a difference between central and peripheral release sites, it may be amplified by modulatory inputs to R15 such as the one from the bag cells. When they fire in a population burst, the bag cells increase the depth of the depolarizing pacemaker waves recorded from the soma of R15 (Mayeri *et al.*, 1979). Thus, within the ganglion, the bag cells may influence release of peptides from R15 by two mechanisms: an increase in spike frequency and modulation of the slower membrane potential trajectory between bursts. The terminals in the periphery, however, are likely to experience only the increase in spike frequency.

Acknowledgments

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